PATENT COOPERATION TREATY

CONFIRMATION

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 060348-0118	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/IB2004/004406	International filing date *(day/month/year)* 8 December 2004	Priority date *(day/month/year)* 9 December 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

A61K 47/00 (2006.01) *C12N 15/09* (2006.01)

Applicant

ENGENEIC MOLECULAR DELIVERY PTY LTD. et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
8 October 2005	27 March 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **CHRIS LUTON** Telephone No. (02) 6283 2256

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/004406

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-46** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **47-50** received by this Authority on **21 March 2006** with the letter of **21 March 2006**
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/7 – 7/7** as originally filed/furnished
pages* received by this Authority on with the letter of
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[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

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* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-35	YES
	Claims		NO
Inventive step (IS)	Claims	1-35	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 – WO 2003/033519
D2 – US 2003/0203481

The present claims define methods for the delivery of nucleic acid molecules to cells by the use of bacterially-derived mincells in combination with bispecific ligands having specificity for a mammalian cell surface receptor capable of activating receptor-mediated endocytosis. The claims also define compositions comprising such minicells and bispecific ligands.

NOVELTY (N) and INVENTIVE STEP (IS)

Neither D1 nor D2 exemplifies the use of bispecific ligands to target minicells containing a therapeutic nucleic acid to non-phagocytic mammalian cells. Therefore, claims 1, 35 and claims dependent therefrom are novel in light of D1 and D2. Neither D1 nor D2 exemplifies a composition comprising minicells with a bispecific ligand capable of binding to both the minicell and a non-phagocytic mammalian cell. Consequently, claim 19 and claims dependent therefrom are novel in light of D1 and D2.

D2 is considered the closest prior art. D2 expressly suggests the use of minicells to deliver gene therapy constructs to mammalian cells (paragraph 697). D2 suggests the use of antibodies and antibody derivatives to target minicells to a cell displaying a membrane protein of choice (paragraph 47, lines 13-16). D2 expressly suggests the use of bispecific ligands (paragraph 106 and paragraph 497, line 13). D2 notes that a variety of binding moieties can be attached to a minicell for the purpose of targeting a particular cell (paragraph 493). D2 suggests targeting cells having an EGF receptor by the use of a ligand to the receptor (paragraph 1107).

Although D2 defines the term "minicell" in broad terms it is quite clear from a reading of the document as a whole that the preferred minicells are anucleate forms of *E. coli* or other bacterial cells, engendered by a disturbance in the coordination, during binary fission, of cell division with DNA segregation. Paragraph 246, Table 2 and the Examples, in particular, clearly indicate the type of minicells preferred by the authors of D2 and those which are enabled by D2.

(continued on extra sheet ...)

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: Box V

D2 provides guidance as to the receptors that can be targeted to cause internalisation by receptor-mediated endocytosis (see paragraphs 645, 650, 678, 699, 735, 736 and 1107). However, D2 teaches the direct targeting of such receptors by proteins expressed on the surface of the minicell rather than by the use of a bispecific ligand specific to both the receptor and the minicell. Therefore, the claims are considered to involve an inventive step in light of D2.

D1 discloses minicells as vectors for DNA transfer and gene therapy and also discloses methods for the purification of minicells. D1 does not suggest the subject matter of the independent claims. Therefore, the claims are considered to involve an inventive step in light of D1.

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8 October 2005	27 March 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer CHRIS LUTON Telephone No. (02) 6283 2256

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

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	Claims		NO
Inventive step (IS)	Claims	1-35	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

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D1 discloses minicells as vectors for DNA transfer and gene therapy and also discloses methods for the purification of minicells. D1 does not suggest the subject matter of the independent claims. Therefore, the claims are considered to involve an inventive step in light of D1.

WHAT IS CLAIMED IS:

1. A targeted gene delivery method that comprises bringing bispecific ligands having specificity for a mammalian cell surface receptor capable of activating receptor-mediated endocytosis into contact with (a) bacterially derived minicells that contain a therapeutic nucleic acid sequence and (b) non-phagocytic mammalian cells, such that (i) said bispecific ligands cause said minicells to bind to said mammalian cells and (ii) said minicells are engulfed by said mammalian cells, which produce an expression product of said therapeutic nucleic acid sequence.

2. A method according to claim 1, wherein said bispecific ligand comprises polypeptide or carbohydrate.

3. A method according to claim 1, wherein said bispecific ligand comprises a first arm that carries specificity for a bacterially derived minicell surface structure and a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

4. A method according to claim 3, wherein said first arm and said second arm are monospecific.

5. A method according to claim 3, wherein said first arm and said second arm are multivalent.

6. A method according to claim 3, wherein said minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on said minicell surface.

7. A method according to claim 3, wherein said minicell surface structure is a member of the group consisting of outer membrane proteins, pilli, fimbrae, flagella, and cell-surface exposed carbohydrates.

8. A method according to claim 3, wherein said mammalian cell surface receptor is capable of activating receptor-mediated endocytosis of said minicell.

9. A method according to claim 1, wherein said bispecific ligand comprises an antibody or antibody fragment.

10. A method according to claim 1, wherein said bispecific ligand comprises a humanized antibody.

11. A method according to claim 1, wherein said minicell comprises an intact cell wall.

12. A method according to claim 1, wherein said therapeutic nucleic acid sequence encodes a suicide gene.

13. A method according to claim 1, wherein said therapeutic nucleic acid encodes a normal counterpart of a gene that expresses a protein that functions abnormally or is present in abnormal levels in said mammalian cells.

14. A method according to claim 1, wherein said mammalian cells are *in vitro*.

15. A method according to claim 1, wherein said mammalian cells are *in vivo*.

16. A method according to claim 1, wherein said therapeutic nucleic acid is contained on a plasmid comprised of multiple nucleic acid sequences.

17. A method according to claim 16, wherein said plasmid comprises a regulatory element.

18. A method according to claim 16, wherein said plasmid comprises a reporter element

19. A composition comprising (i) a bacterially derived minicell that contains a therapeutic nucleic acid molecule and (ii) a bispecific ligand that is capable of binding to a surface component of said minicell and to a surface component of a non-phagocytic mammalian cell, wherein said bispecific ligand has specificity for a mammalian cell surface receptor capable of activating receptor-mediated endocytosis.

20. The composition of claim 19, wherein said bispecific ligand comprises polypeptide or carbohydrate.

21. The composition of claim 19, wherein said bispecific ligand comprises a first arm that carries specificity for a bacterially derived minicell surface structure and a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

22. The composition of claim 21, wherein said first arm and said second arm are monospecific.

23. The composition of claim 21, wherein said first arm and said second arm are multivalent.

24. The composition of claim 21, wherein said minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on said minicell surface.

25. The method of claim 21, wherein said minicell surface structure is a member of the group consisting of outer membrane proteins, pilli, fimbrae, flagella, and cell-surface exposed carbohydrates.

26. The composition of claim 21, wherein said mammalian cell surface receptor is capable of activating receptor-mediated endocytosis of said minicell.

27. The composition of claim 19, wherein said bispecific ligand comprises an antibody or antibody fragment.

28. The composition of claim 19, wherein said bispecific ligand comprises a humanized antibody.

29. The composition of claim 19, wherein said minicell comprises an intact cell wall.

30. The composition of claim 19, wherein said therapeutic nucleic acid sequence encodes a suicide gene.

31. The composition of claim 19, wherein said therapeutic nucleic acid encodes a normal counterpart of a gene that expresses a protein that functions abnormally or is present in abnormal levels in said mammalian cell.

32. The composition of claim 19, wherein said therapeutic nucleic acid is contained on a plasmid comprised of multiple nucleic acid sequences.

33. The composition of claim 32, wherein said plasmid comprises a regulatory element.

34. The composition of claim 32, wherein said plasmid comprises a reporter element.

35. Use of bacterially derived intact minicells and bispecific ligands in the preparation of a medicament, said minicells containing a therapeutic nucleic acid molecule and said bispecific ligands being capable of binding to said minicells and to a receptor capable of activating receptor-mediated endocytosis on target non-phagocytic mammalian cells, for use in a method of treating a disease or modifying a trait by administration of said medicament to a cell, tissue, or organ.